AD_____

Award Number: W81XWH-13-1-0029

TITLE: Regulation of Survival by IKKE in Inflammatory Breast Cancer Involves EpCAM

PRINCIPAL INVESTIGATOR: Thanh Barbie, MD

CONTRACTING ORGANIZATION: Washington University
St. Louis, MO 63110-1010

REPORT DATE: July 2014

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release; Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2014	Annual	1Dec2013 - 30Jun2014

4. TITLE AND SUBTITLE
Regulation of Survival by IKKe in Inflammatory Breast Cancer Involves EpCAM

5a. CONTRACT NUMBER
W81XWH-13-1-0029

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Barbie, Thanh

E-Mail:barbiet@wudosis.wustl.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Washington University
The One Brookings Dr.
St. Louis, MO 63130-4862

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Although triple negative breast cancers (TNBC) consistently lack hormone receptor expression and ERBB2 amplification, several lines of evidence suggest that these cancers are heterogeneous. Here we find that aberrant expression of the I κ B kinase (IKK) related-kinase IKK ε drives a specific subset of TNBC that are maintained by an autocrine cytokine circuit involving JAK/STAT pathway activation. We identify CYT387 as a novel potent inhibitor of IKK ε and JAK signaling that disrupts this circuit and preferentially impairs the proliferation of IKK ε -driven breast cancer cells in vitro. CYT387 treatment inhibits both NF- κ B and STAT activation and disrupts expression of the pro-tumorigenic cytokines CCL5 and IL-6 in these breast cancer cells. Interruption of cytokine signaling by CYT387 in vivo impairs the growth of an IKK ε -driven TNBC cell line and patient-derived xenografts. These findings elucidate a specific immune-driven subtype of TNBC that is sensitive to combined IKK ε and JAK inhibition.

15. SUBJECT TERMS
Triple Negative Breast Cancer, EpCAM, IKKε, IKKε small molecule inhibitor

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	12	

Table of Contents

INTRODUCTION

Breast cancer is the leading cause of cancer incidence and second leading cause of cancer deaths in women in the United States (*1*). Despite tremendous advances in screening, surgical management, and targeted therapies such as endocrine and HER2-directed treatments, the prognosis for women with advanced disease remains poor.

Epithelial cell adhesion molecule (EpCAM) is a transmembrane glycoprotein that is expressed on normal epithelial cells and over-expressed in a subset of carcinomas, including breast and ovarian cancer (*2*). It has attracted recent attention as a tool for capture-based detection of circulating cells (*3*), as well as a marker for stem cell-like tumor initiating cells (*4*). Emerging evidence from the Gillanders' laboratory also supports the concept that EpCAM is not simply a passive cell surface marker, but rather actively regulates breast cancer proliferation and invasion (5, 6).

To gain even further insight into EpCAM regulation and function, we first explored *EPCAM* gene expression across a panel of 1062 primary breast cancers (*5-7*) and cell lines (http://www.broadinstitute.org/ccle/home) to identify the specific molecular subtypes of breast cancer in which it is over-expressed. This analysis showed that *EPCAM* expression was enriched in an inflammatory subtype of triple negative breast cancer. Interestingly, this subtype of breast cancer is characterized by over-expression of several immune associated genes, including the non-canonical IκB kinase *IKBKE* (encoding IKKε). IKKε and its homologue TBK1represent an emerging link between inflammation and cancer (*8*). IKKε is overexpressed and/or amplified in approximately 30% of breast carcinomas (*9-11*), where it induces survival signaling associated with NF-κB pathway activation. Aberrant IKKε expression facilitates cell transformation, whereas suppression of IKKε in breast cancer cell lines that harbor IKKε amplification results in cell death (*10*). IKKε phosphorylates CYLD and TRAF2 in breast cancer cells, which contributes to NF-κB activation and promotes tumorigenesis (*12, 13*). IKKε also directly phosphorylates and activates specific STAT transcription factors (*14, 15*). Furthermore, cytokines produced by TBK1/IKKε can engage downstream JAK/STAT signaling in an autocrine or paracrine fashion (*16*).

Activation of NF-κB and JAK/STAT signaling has also been strongly implicated in this subtype of TNBC (*17-21*). IKKε coordinately activates NF-κB and STAT signaling in these cells and sustains protumorigenic cytokine production. CYT387, a dual TBK1/IKKε and JAK inhibitor, potently disrupts this inflammatory signaling circuit and impairs tumor progression in preclinical mouse models of TNBC, identifying a novel therapeutic strategy for this refractory breast cancer subtype.

BODY

Specific Aim 1: Define the mechanism(s) by which EpCAM is regulated by IKKε.

In addition to being overexpressed in a subset of luminal/ER+ breast cancers as previously reported (*10*), we also found that IKKε mRNA was highly expressed in this EpCAM positive subset of ER- breast cancers and particularly in TNBC (Figure 1A). Induction of IKKε mRNA in this subset of TNBC tumors was more closely associated with IL-1 pathway activation, as evidenced by co-expression of an IL-1 signature (*22*) (Fig. 1A). Hierarchical clustering with previously reported gene expression subtypes (*23*) further revealed that IKKε expression and IL-1 activation were associated most closely with the immunomodulatory subtype of TNBC (data not shown). We next identified TNBC cell lines with elevated IKKε levels using gene-expression data from the Broad/Novartis Cell Line Encyclopedia (*24*) and validated that these cell lines expressed high levels of IKKε protein (Fig 1B, C). Using two independent IKKε-specific shRNAs, we found that the TNBC cell lines MDA-MB-468 cells and MDA-MB-231 were sensitive to suppression of IKKε, whereas specific ablation of IKKε failed to affect the proliferation of non-transformed MCF10A cells (Fig. 1B). These findings revealed that IKKε is not only overexpressed, but also contributes to the proliferation and survival of this subset of TNBC.

When we examined the relationship between IKKε and STAT3 activation (as measured by Y705 pSTAT3 levels), we observed a strong correlation between elevated IKKε levels and activated STAT3 in TNBC cell lines (Fig. 1C). Further, IKKε overexpression in HEK-293T cells not only induced NF-κB pathway activation as measured by S933 pNF-κB p105
levels (Fig. 1D), as well as CCL5 and IL-6 expression (data not shown). Taken together, these findings confirm that IKKε signaling promotes NF-κB, STAT3 and cytokine activation.

Despite our preliminary observations that EpCAM was linked with IKKε expression in this subtype of TNBC, subsequent experiments following IKKε suppression or over-expression failed to give consistent results. While we still suspect that EpCAM expression in these tumors reflects epithelial differentiation within this inflammatory subset of tumors, it is not clear that the relationship with IKKε is direct. Although we remain interested in the specific features that delineate this tumor cell state as a means to identify predictive biomarkers, our focus has shifted to Aims #2 and #3, in which we have made significant progress towards a novel therapeutic strategy for this breast cancer subtype.

Specific Aim 2: Define the ability of small molecule inhibitors of IKKε to inhibit breast cancer growth and invasion.

Since IKKε expressing TNBC cells exhibited STAT3 activation, we considered the possibility that inhibition of JAK/STAT signaling by treatment with the clinically advanced JAK inhibitors Ruxolitinib (*25*) or CYT387 (*26*) might impact their proliferation and survival.

Treatment of MDA-MB-468 cells with several different doses of Ruxolitinib or CYT387 inhibited STAT3 phosphorylation (Fig. 1E). However, when we treated multiple different cell lines with 5 μM Ruxolitinib, which completely inhibited pSTAT3, we failed to observe any effect on cell viability in contrast to CYT387 (Fig. 1F, G). These findings suggested an additional activity of CYT387.

Since CYT387 inhibits the IKKε homologue TBK1 (*27*), we next assessed whether IKKε signaling was inhibited by CYT387. Both CYT387 and Ruxolitinib inhibited IKKε-induced Y705 pSTAT3 (Fig. 2A). However, CYT387 alone inhibited IKKε-induced NF-κB (Fig. 2B) and also directly impaired IKKε expression itself (Fig. 2C). We also collected media from 293T cells following transfection with EGFP or IKKε and analyzed levels of 36 different cytokines and chemokines using a antibody array. Expression of IKKε potently induced CCL5 levels in the media, which was completely abrogated by CYT387 but not Ruxolitinib treatment (Fig. 2D). We confirmed by ELISA that IKKε-induced CCL5 and IL-6 were preferentially inhibited by CYT387 (data not shown). Thus, the unique activity of CYT387 in IKKε-driven TNBC relates to its activity as a TBK1/IKKε inhibitor.

Specific Aim 3: Evaluation of IKBKE small molecule inhibitors in *vivo* using a patient tumor-derived breast cancer xenograft model (HAMLET: Human and Mouse Linked Evaluation of Tumors).

Since CYT387 has proven to be safe in both mice and humans (*26, 27*), we next explored the therapeutic impact of CYT387 therapy in clinically relevant models *in vivo*. After tumors were established in nude mice, CYT387 was administered via daily oral gavage at a dose of 100 mg/kg. CYT387 treatment impaired the growth of established MDA-MB-468 xenografts, as well as two different Washington University Human-in Mouse (WHIM) lines (WHIM4 and WHIM21) derived from patients with IKKε expressing TNBC (Fig. 2E, data not

shown). CYT387 treatment suppressed IKKε expression in WHIM21 patient derived xenografts *in vivo,* potently inhibited *CCL5* and *IL-6* expression, and suppressed activated STAT3 (Fig 2G, data not shown). Thus, CYT387 effectively inhibits IKKε and JAK signaling in vivo, suppresses protumorigenic cytokine expression, and exhibits therapeutic potential for IKKε-driven TNBC.

Since CYT387 inhibits IKK□ and JAK and is effective as a single agent in TNBC, we considered it might synergize even more potently with inhibitors of PI3K/mTOR or MEK/ERK signaling (*28*). While CYT387 inhibits IKKε and JAK and is effective as a single agent in TNBC, we considered further synergy with inhibitors of MEK/ERK signaling. We focused specifically on MEK inhibition since monotherapy results in dynamic reprogramming of the kinome involving receptor tyrosine kinase (RTK) and cytokine activation. We speculated that treatment of TNBCs with trametinib may prime these breast cancer cells to become more dependent on the JAK/STAT pathway and cytokines and therefore treatment with CYT387 would result in a more robust response. We first confirmed that dual CYT387 (momelotinib) and MEK inhibitor GSK112012 (trametinib) treatment effectively inhibited both pERK and pSTAT3 levels (data not shown). Consistent with our hypothesis, tumor growth was dramatically impaired in an aggressive TNBC PDX model (WHIM21), (Fig. 3A, B). In addition to WHIM21, another *in vivo* model of TNBC with high IKKε expression (WHIM4) shows a similar drastic response to combination therapy (Fig. 4A). These WHIM models were obtained from patients who succumbed to their disease after multiple chemotherapies. Thus, momelotinib/trametinib may represent a highly effective combination therapy for chemotherapy resistant TNBCs. Furthermore, in preclinical studies, these WHIM models had been used to evaluate the drug efficacy of several targeted therapies in development. Unlike the drastic impairment in tumor growth seen with our combination regimen of CYT387/trametinib, the other targeted therapies used resulted in no or minimal growth inhibitory effects. In addition, combination treatment of a TNBC xenograft with low IKK □ exp inhibitory effect (Fig. 4B). This is likely secondary to the reprogramming of the kinome that results from MEK inhibition, rendering these breast cancer cells more sensitive to the effects of cytokines. As such, this combination therapy may have a broader efficacy for TNBCs aside from the immunomodulatory subtype. The specificity of this regimen to TNBCs is highlighted by the fact that no growth response is observed when this combination therapy was used to treat the WHIM20 xenograft, which is a luminal breast cancer with low IKK □ expression (Fig. 4C).

In addition to a significant decrease in size of the TNBC xenografts treated with CYT387/trametinib, the tumors also appeared particularly pale compared with vehicle or single agent treated tumors (Fig. 2G, 3B, 4A, 4B). We are therefore in the process of performing a detailed histologic examination of the WHIM21 treated tumors, including measures of angiogenesis. Combined CYT387/trametinib treatment impairs tumor progression and perhaps angiogenesis, representing a promising novel therapy for TNBC.

KEY RESEARCH ACCOMPLISHMENTS

- Identified IKKε as a novel driver of an inflammatory subtype of TNBC that maintains features of epithelial differentiation
- Characterized specific NF-κB, STAT3, and cytokine signaling pathways that contribute to IKKε mediated tumorigenesis
- Discovered CYT387 as a potent IKKε and JAK inhibitor that inhibits this breast cancer subtype *in vitro*
- Identified therapeutic activity of CYT387 in IKKε driven TNBC patient derived xenografts
- Discovered that CYT387 in combination therapy with the MEK inhibitor (Trametinib) results in significant growth inhibition of patient-derived xenografts

REPORTABLE OUTCOMES

Poster Award: "CYT387 as a Novel Treatment for Triple Negative Breast Cancer." Washington University Breast Cancer Retreat – Award Winning Poster (2013).

Manuscript submitted to *Journal of Clinical Investigation*: Barbie TU, Alexe G, Zhu Z, Aref A, Li S, Thai T, Imamura Y, Zhang X, Cohoon TJ, Herndon J, Fleming T, Ogino S, Wong KK, Ellis MJ, Hahn WC, Barbie DA, Gillanders WE. Targeting an IKBKE cytokine network impairs triple-negative breast cancer growth.

CONCLUSIONS

In summary, the initial focus on EpCAM has led us to identify a novel subset of TNBC that depends on IKKε expression and is sensitive to treatment with CYT387. We have identified a specific TNBC subset characterized by aberrant expression of the IKK-related kinase IKKε and production of protumorigenic cytokines CCL5 and IL-6. These tumors show substantial overlap with the immunomodulatory (IM) subtype of TNBC, recently identified by gene expression profiling studies (*23*). These triple negative tumors exhibit inducible IKKε expression associated with markers of IL-1 signaling. In addition, despite engagement of the JAK-STAT pathway, treatment with the potent and selective JAK1/2 inhibitor Ruxolitinib was insufficient to impair viability of these TNBC. Instead, another clinical stage JAK inhibitor, CYT387, impaired the proliferation of TNBC cells *in vitro* and *in vivo.* The efficacy of CYT387 was directly related to its additional ability to inhibit IKKε activity and the production of pro-tumorigenic cytokines, CCL5 and/or IL-6. These observations suggest a promising therapeutic option for a subset of patients with IKKε driven TNBC.

Clinical trials of selective JAK1/2 inhibitors such as Ruxolitinib have entered clinical evaluation for breast cancer. While JAK-STAT signaling is clearly active in this subset of TNBC, our data suggests that JAK inhibition alone may not be sufficient to disrupt this circuit. Furthermore, although certain markers such as $CD44^{+}CD24^{-}$ positivity or the IM gene expression profile have been associated with this particular TNBC phenotype, the underlying driver of cytokine activation in these cancers has remained elusive. The identification of IKKε as a key driver of this cytokine-signaling network not only provides an additional marker of this emerging TNBC subtype, but also a discrete molecular target. Our data suggests that the capacity of CYT387 to inhibit both IKKε and JAK/STAT signaling, resulting in a particularly potent anti-cytokine effect, may yield superior clinical activity in TNBC relative to more selective JAK1/2 inhibitors. Finally, since dual inhibition of IKKε and JAK by CYT387 is already effective as a single agent in TNBC, it is thus possible that this drug may synergize even more potently with inhibitors of PI3K/mTOR or other pathways such as MEK/ERK signaling. Future studies will be focused on further elucidating the mechanism of IKKε and on performing additional xenograft studies with CYT387 drug combinations.

I am sincerely grateful for the DOD Breast Cancer Research Postdoctoral Fellowship Award, which has supported my development as a physician scientist. Obtaining expertise in the laboratory and as an academic breast cancer surgeon will enable me to promote the translation of novel targeted therapies to the clinic. I am truly excited about the next phase of my career and the potential to have an impact on the lives of women suffering with breast cancer.

REFERENCES

1. A. Jemal, R. Siegel, J. Xu, E. Ward, Cancer statistics, 2010. *CA: a cancer journal for clinicians* **60**, 277-300 (2010).
2. B. T. van der Gun, L. J. Melchers, M. H. Ruiters, L. F. de Leij, P. M. McLaughlin, M. G. Rots, EpCAM in carcinogenesis: the good, the bad or the ugly. *Carcinogenesis* **31**, 1913-1921 (2010).
3. S. Maheswaran, D. A. Haber, Circulating tumor cells: a window into cancer biology and metastasis. *Current opinion in genetics & development* **20**, 96-99 (2010).
4. J. E. Visvader, G. J. Lindeman, Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nature reviews. Cancer* **8**, 755-768 (2008).
5. Y. Wang, J. G. Klijn, Y. Zhang, A. M. Sieuwerts, M. P. Look, F. Yang, D. Talantov, M. Timmermans, M. E. Meijer-van Gelder, J. Yu, T. Jatkoe, E. M. Berns, D. Atkins, J. A. Foekens, Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. *Lancet* **365**, 671-679 (2005).
6. A. V. Ivshina, J. George, O. Senko, B. Mow, T. C. Putti, J. Smeds, T. Lindahl, Y. Pawitan, P. Hall, H. Nordgren, J. E. Wong, E. T. Liu, J. Bergh, V. A. Kuznetsov, L. D. Miller, Genetic reclassification of histologic grade delineates new clinical subtypes of breast cancer. *Cancer research* **66**, 10292-10301 (2006).
7. C. Desmedt, F. Piette, S. Loi, Y. Wang, F. Lallemand, B. Haibe-Kains, G. Viale, M. Delorenzi, Y. Zhang, M. S. d'Assignies, J. Bergh, R. Lidereau, P. Ellis, A. L. Harris, J. G. Klijn, J. A. Foekens, F. Cardoso, M. J. Piccart, M. Buyse, C. Sotiriou, Strong time dependence of the 76-gene prognostic signature for node-negative breast cancer patients in the TRANSBIG multicenter independent validation series. *Clinical cancer research : an official journal of the American Association for Cancer Research* **13**, 3207-3214 (2007).
8. R. R. Shen, W. C. Hahn, Emerging roles for the non-canonical IKKs in cancer. *Oncogene* **30**, 631-641 (2011).
9. M. Adli, A. S. Baldwin, IKK-i/IKKepsilon controls constitutive, cancer cell-associated NF-kappaB activity via regulation of Ser-536 p65/RelA phosphorylation. *The Journal of biological chemistry* **281**, 26976-26984 (2006).
10. J. S. Boehm, J. J. Zhao, J. Yao, S. Y. Kim, R. Firestein, I. F. Dunn, S. K. Sjostrom, L. A. Garraway, S. Weremowicz, A. L. Richardson, H. Greulich, C. J. Stewart, L. A. Mulvey, R. R. Shen, L. Ambrogio, T. Hirozane-Kishikawa, D. E. Hill, M. Vidal, M. Meyerson, J. K. Grenier, G. Hinkle, D. E. Root, T. M. Roberts, E. S. Lander, K. Polyak, W. C. Hahn, Integrative genomic approaches identify IKBKE as a breast cancer oncogene. *Cell* **129**, 1065-1079 (2007).
11. S. F. Eddy, S. Guo, E. G. Demicco, R. Romieu-Mourez, E. Landesman-Bollag, D. C. Seldin, G. E. Sonenshein, Inducible IkappaB kinase/IkappaB kinase epsilon expression is induced by CK2 and promotes aberrant nuclear factor-kappaB activation in breast cancer cells. *Cancer research* **65**, 11375-11383 (2005).
12. J. E. Hutti, R. R. Shen, D. W. Abbott, A. Y. Zhou, K. M. Sprott, J. M. Asara, W. C. Hahn, L. C. Cantley, Phosphorylation of the tumor suppressor CYLD by the breast cancer oncogene IKKepsilon promotes cell transformation. *Molecular cell* **34**, 461-472 (2009).
13. R. R. Shen, A. Y. Zhou, E. Kim, E. Lim, H. Habelhah, W. C. Hahn, IkappaB kinase epsilon phosphorylates TRAF2 to promote mammary epithelial cell transformation. *Molecular and cellular biology* **32**, 4756-4768 (2012).
14. B. R. Tenoever, S. L. Ng, M. A. Chua, S. M. McWhirter, A. Garcia-Sastre, T. Maniatis, Multiple functions of the IKK-related kinase IKKepsilon in interferon-mediated antiviral immunity. *Science* **315**, 1274-1278 (2007).
15. S. L. Ng, B. A. Friedman, S. Schmid, J. Gertz, R. M. Myers, B. R. Tenoever, T. Maniatis, IkappaB kinase epsilon (IKK(epsilon)) regulates the balance between type I and type II interferon responses. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 21170-21175 (2011).
16. S. Sankar, H. Chan, W. J. Romanow, J. Li, R. J. Bates, IKK-i signals through IRF3 and NFkappaB to mediate the production of inflammatory cytokines. *Cellular signalling* **18**, 982-993 (2006).
17. L. Li, P. E. Shaw, Autocrine-mediated activation of STAT3 correlates with cell proliferation in breast carcinoma lines. *The Journal of biological chemistry* **277**, 17397-17405 (2002).

18. M. Berishaj, S. P. Gao, S. Ahmed, K. Leslie, H. Al-Ahmadie, W. L. Gerald, W. Bornmann, J. F. Bromberg, Stat3 is tyrosine-phosphorylated through the interleukin-6/glycoprotein 130/Janus kinase pathway in breast cancer. *Breast cancer research : BCR* **9**, R32 (2007).
19. S. R. Walker, E. A. Nelson, L. Zou, M. Chaudhury, S. Signoretti, A. Richardson, D. A. Frank, Reciprocal effects of STAT5 and STAT3 in breast cancer. *Molecular cancer research : MCR* **7**, 966-976 (2009).
20. M. Hedvat, D. Huszar, A. Herrmann, J. M. Gozgit, A. Schroeder, A. Sheehy, R. Buettner, D. Proia, C. M. Kowolik, H. Xin, B. Armstrong, G. Bebernitz, S. Weng, L. Wang, M. Ye, K. McEachern, H. Chen, D. Morosini, K. Bell, M. Alimzhanov, S. Ioannidis, P. McCoon, Z. A. Cao, H. Yu, R. Jove, M. Zinda, The JAK2 inhibitor AZD1480 potently blocks Stat3 signaling and oncogenesis in solid tumors. *Cancer cell* **16**, 487-497 (2009).
21. L. L. Marotta, V. Almendro, A. Marusyk, M. Shipitsin, J. Schemme, S. R. Walker, N. Bloushtain-Qimron, J. J. Kim, S. A. Choudhury, R. Maruyama, Z. Wu, M. Gonen, L. A. Mulvey, M. O. Bessarabova, S. J. Huh, S. J. Silver, S. Y. Kim, S. Y. Park, H. E. Lee, K. S. Anderson, A. L. Richardson, T. Nikolskaya, Y. Nikolsky, X. S. Liu, D. E. Root, W. C. Hahn, D. A. Frank, K. Polyak, The JAK2/STAT3 signaling pathway is required for growth of CD44CD24 stem cell-like breast cancer cells in human tumors. *The Journal of clinical investigation* **121**, 2723-2735 (2011).
22. J. Jura, P. Wegrzyn, M. Korostynski, K. Guzik, M. Oczko-Wojciechowska, M. Jarzab, M. Kowalska, M. Piechota, R. Przewlocki, A. Koj, Identification of interleukin-1 and interleukin-6-responsive genes in human monocyte-derived macrophages using microarrays. *Biochimica et biophysica acta* **1779**, 383-389 (2008).
23. B. D. Lehmann, J. A. Bauer, X. Chen, M. E. Sanders, A. B. Chakravarthy, Y. Shyr, J. A. Pietenpol, Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *The Journal of clinical investigation* **121**, 2750-2767 (2011).
24. J. Barretina, G. Caponigro, N. Stransky, K. Venkatesan, A. A. Margolin, S. Kim, C. J. Wilson, J. Lehar, G. V. Kryukov, D. Sonkin, A. Reddy, M. Liu, L. Murray, M. F. Berger, J. E. Monahan, P. Morais, J. Meltzer, A. Korejwa, J. Jane-Valbuena, F. A. Mapa, J. Thibault, E. Bric-Furlong, P. Raman, A. Shipway, I. H. Engels, J. Cheng, G. K. Yu, J. Yu, P. Aspesi, Jr., M. de Silva, K. Jagtap, M. D. Jones, L. Wang, C. Hatton, E. Palescandolo, S. Gupta, S. Mahan, C. Sougnez, R. C. Onofrio, T. Liefeld, L. MacConaill, W. Winckler, M. Reich, N. Li, J. P. Mesirov, S. B. Gabriel, G. Getz, K. Ardlie, V. Chan, V. E. Myer, B. L. Weber, J. Porter, M. Warmuth, P. Finan, J. L. Harris, M. Meyerson, T. R. Golub, M. P. Morrissey, W. R. Sellers, R. Schlegel, L. A. Garraway, The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* **483**, 603-607 (2012).
25. S. Verstovsek, R. A. Mesa, J. Gotlib, R. S. Levy, V. Gupta, J. F. DiPersio, J. V. Catalano, M. Deininger, C. Miller, R. T. Silver, M. Talpaz, E. F. Winton, J. H. Harvey, Jr., M. O. Arcasoy, E. Hexner, R. M. Lyons, R. Paquette, A. Raza, K. Vaddi, S. Erickson-Viitanen, I. L. Koumenis, W. Sun, V. Sandor, H. M. Kantarjian, A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. *The New England journal of medicine* **366**, 799-807 (2012).
26. A. Pardanani, R. R. Laborde, T. L. Lasho, C. Finke, K. Begna, A. Al-Kali, W. J. Hogan, M. R. Litzow, A. Leontovich, M. Kowalski, A. Tefferi, Safety and efficacy of CYT387, a JAK1 and JAK2 inhibitor, in myelofibrosis. *Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K* **27**, 1322-1327 (2013).
27. J. W. Tyner, T. G. Bumm, J. Deininger, L. Wood, K. J. Aichberger, M. M. Loriaux, B. J. Druker, C. J. Burns, E. Fantino, M. W. Deininger, CYT387, a novel JAK2 inhibitor, induces hematologic responses and normalizes inflammatory cytokines in murine myeloproliferative neoplasms. *Blood* **115**, 5232-5240 (2010).
28. J. S. Duncan, M. C. Whittle, K. Nakamura, A. N. Abell, A. A. Midland, J. S. Zawistowski, N. L. Johnson, D. A. Granger, N. V. Jordan, D. B. Darr, J. Usary, P. F. Kuan, D. M. Smalley, B. Major, X. He, K. A. Hoadley, B. Zhou, N. E. Sharpless, C. M. Perou, W. Y. Kim, S. M. Gomez, X. Chen, J. Jin, S. V. Frye, H. S. Earp, L. M. Graves, G. L. Johnson, Dynamic reprogramming of the kinome in response to targeted MEK inhibition in triple-negative breast cancer. *Cell* **149**, 307-321 (2012).

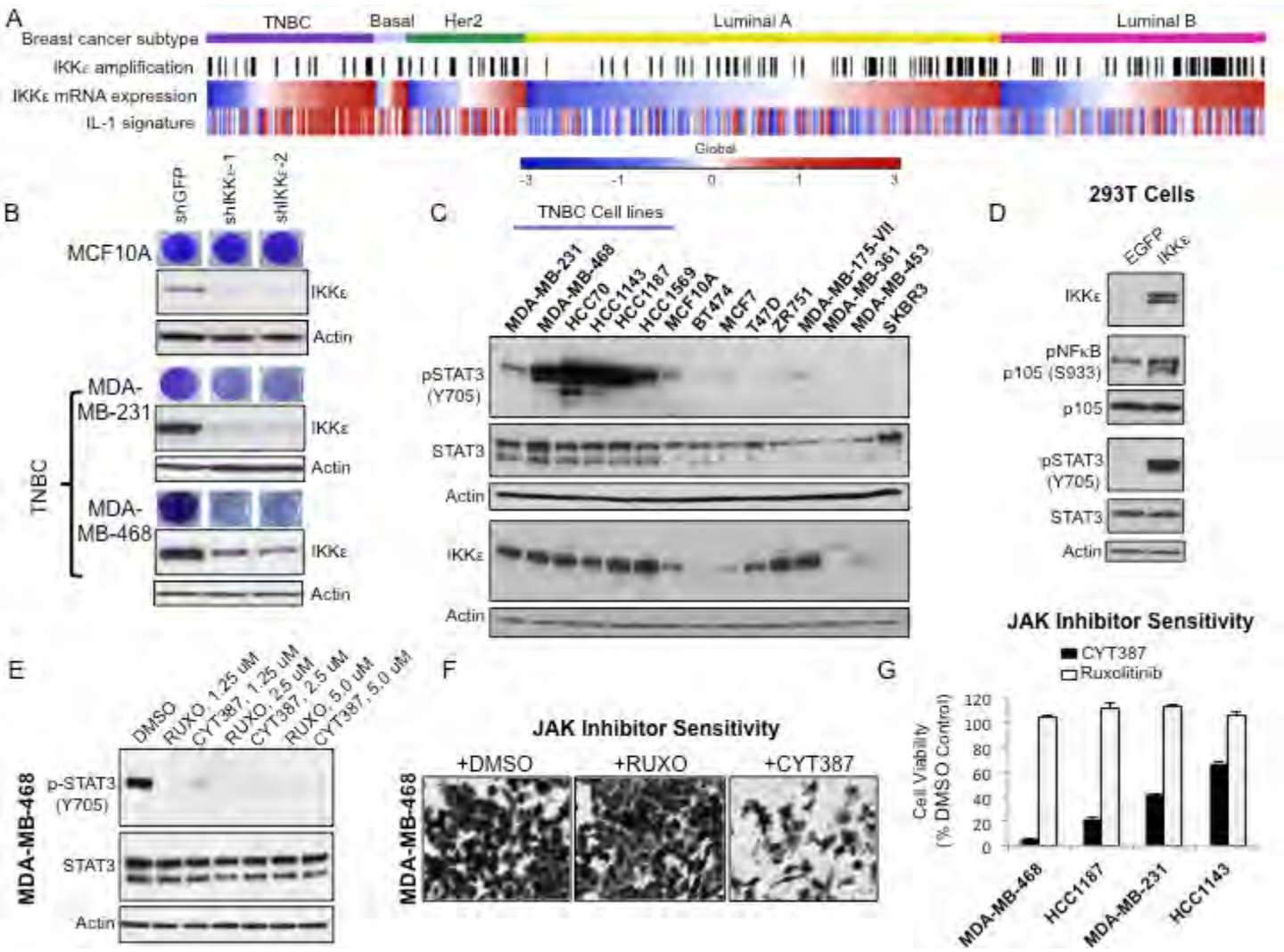

Figure 1. A. Heatmap of IKKε mRNA levels vs amplification or IL-1 signature expression in TCGA breast tumor data. **B.** TNBC cell line dependence on IKKε expression. **C.** STAT3 activation correlates with IKKε over-expression in TNBC cell lines. **D.** IKKε directly induces NF-κB and STAT3 activation. **E.** Ruxolitinib or CYT387 treatment inhibits STAT3 activation in TNBC cells. **F.** Phase contrast images of TNBC cells treated with 5 μM Ruxolitinib or CYT387. **G.** Cell viability data following JAK inhibitor treatment of TNBC cells.

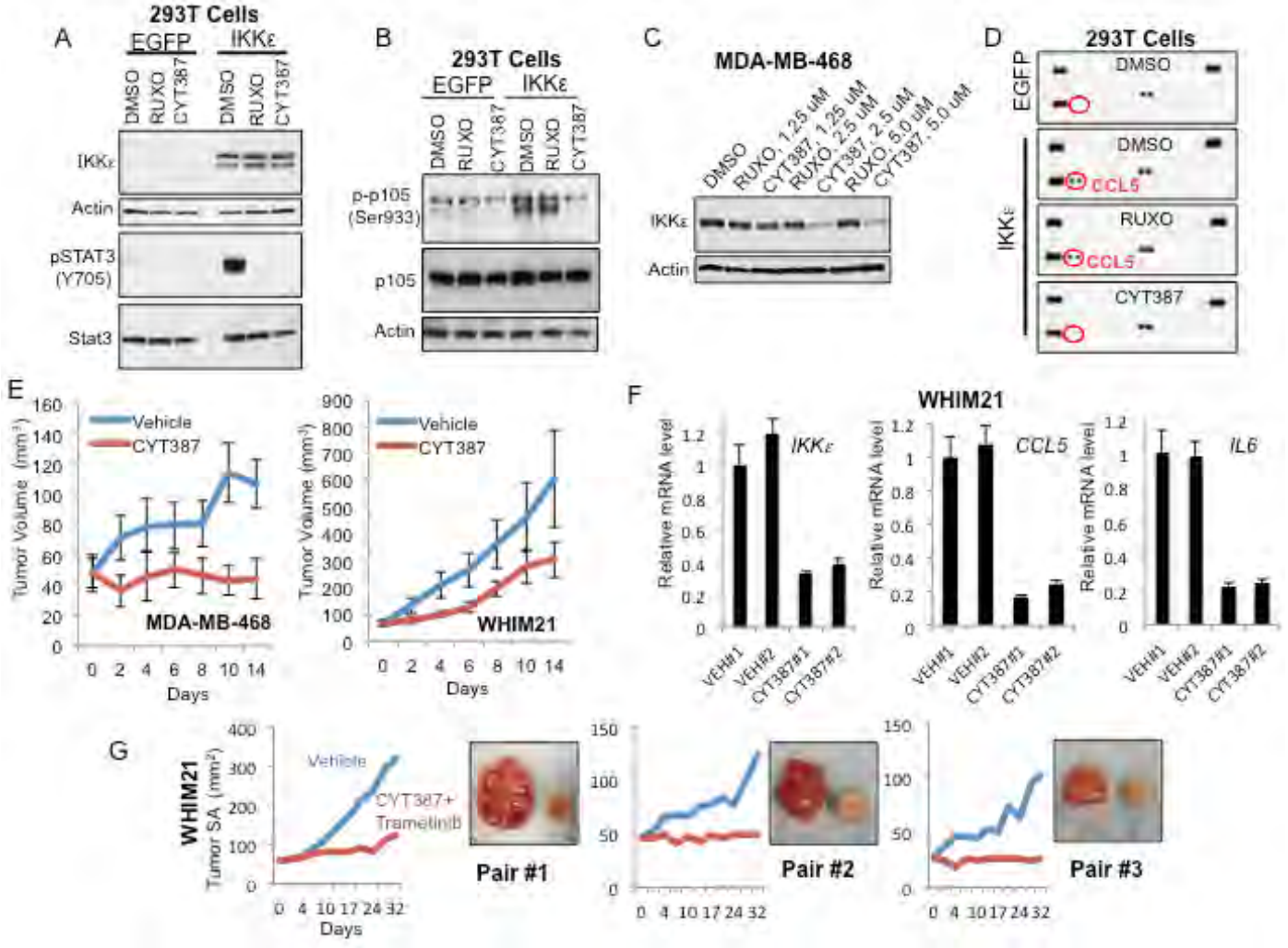

Figure 2. A. Immuno-blot of IKKε, pSTAT3, STAT3, and Actin levels following EGFP or IKKε expression and inhibitor treatment of 293T cells. **B.** Immunoblot of p-pl05, p105 and Actin levels following EGFP or IKKε expression and inhibitor treatment of 293T cells. **C.** IKKε and Actin levels following treatment of TNBC cells with inhibitors. **D.** Cytokine levels of 293T cell media following IKKε expression and inhibitor treatment. **E**. Effects of CYT387 treatment on cell-line or patient-derived TNBC xenografts. **F.** IKKε, CCL5, and IL-6 levels in treated tumors. **G.** Effects of combination CYT387 and trametinib therapy on TNBC PDX growth.

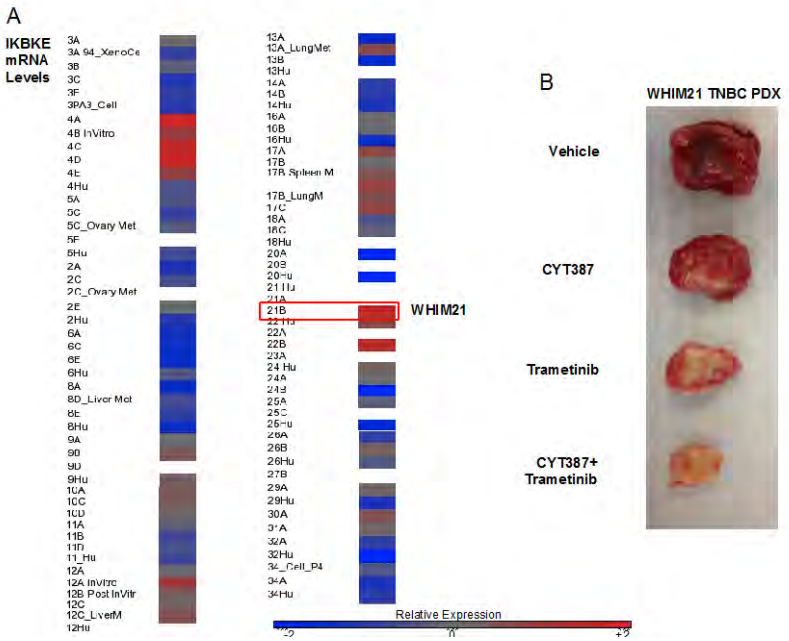

Figure 3. A. Identification of a Washington University human-in-mouse line (WHIM21) that was derived from a patient with a TNBC that overexpressed IKKε. **B.** Established WHIM21 tumors were then treated with vehicle only, CYT387 at 50mg/kg/d, Trametinib at 2.5 mg/kg/d, or combination therapy with CYT387 and trametinib.

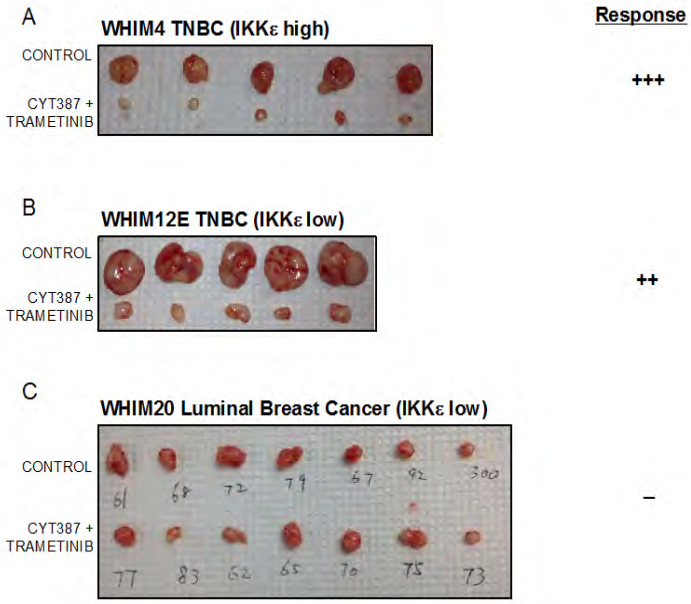

Figure 4. A. Established WHIM4 (TNBC with high IKKε expression) were treated with control or vehicle only versus combination therapy with CYT387/trametinib. **B.** Established WHIM12E (TNBC with low IKKε expression) were treated with vehicle only versus combination therapy with CYT387/trametinib. **C.** Established WHIM20 (Luminal breast cancer with low IKKε expression) were treated with vehicle only versus combination therapy with CYT387/trametinib.